

(19)



Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number:

**0 300 735
A1**

(12)

EUROPEAN PATENT APPLICATION

(21) Application number: 88306598.9

(22) Date of filing: 19.07.88

(91) Int. Cl. 4: **A61K 31/65 , //(A61K31/65,
31:435),(A61K31/65,31:495),
(A61K31/65,31:47)**

(30) Priority: 20.07.87 HU 333587

(43) Date of publication of application:
25.01.89 Bulletin 89/04

(84) Designated Contracting States:
AT BE CH DE ES FR GB GR IT LI LU NL SE

(71) Applicant: **CHINOIN Gyogyszer és Vegyészeti
Termékek Gyára RT.**
To utca 1-5
H-1045 Budapest IV(HU)

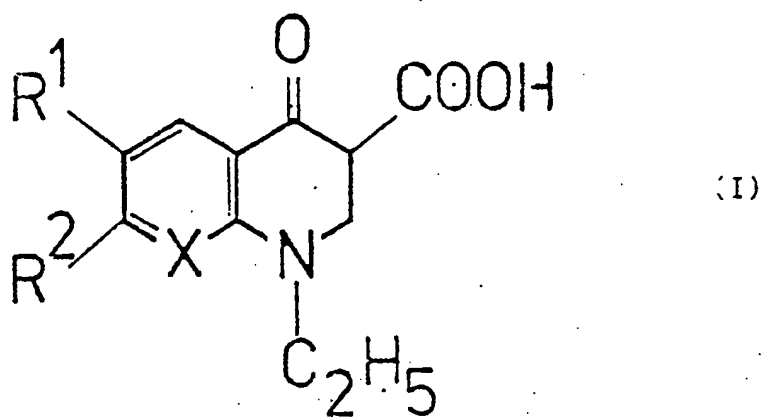
(72) Inventor: **Kulcsar, Gabor**
138 Szlovak Utca
HU-1162 Budapest(HU)
Inventor: **Sarkozy, Peter**
10 Varfok Utca
HU-1012 Budapest(HU)
Inventor: **Frank, Judit**
2 Szolo Koz
HU-1032 Budapest(HU)
Inventor: **Kaloy, Katalin**
15 Ibrahim Utca
HU-1113 Budapest(HU)

(74) Representative: **Skalles, Humphrey John et al**
Frank B. Dehn & Co. Imperial House 15-19
Kingsway
London WC2B 6UZ(GB)

(54) **Antimicrobial pharmaceutical composition.**

(57) The invention relates to a synergistic, anti-microbial pharmaceutical composition containing 0.01 to 50 % by weight of a quinolinecarboxylic acid derivative or a naphthyridinecarboxylic acid derivative of the general formula (I).

EP 0 300 735 A1



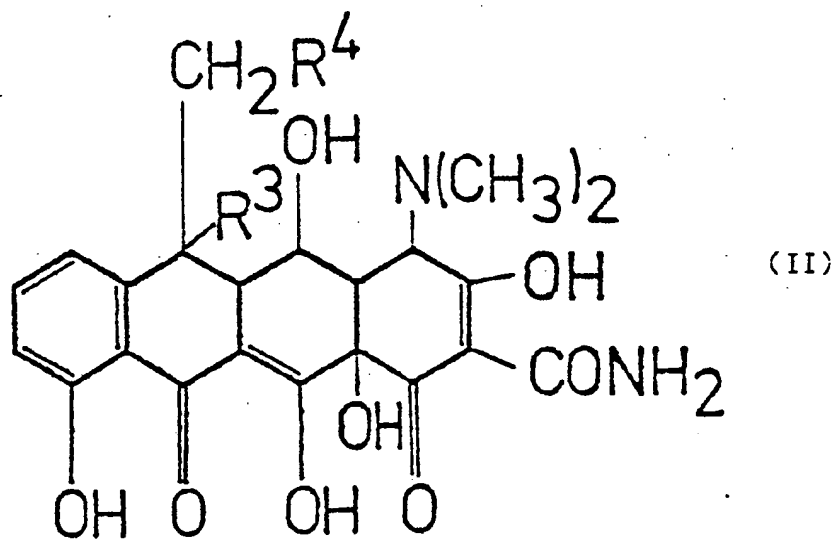
wherein

X is carbon or nitrogen;

R¹ is hydrogen or fluorine;

R² is methyl, piperazino or methylpiperazino group; or

R¹ and R² together are a methylenedioxy group; and 0.01 to 95 % by weight of a tetracycline derivative of the general formula (II).



wherein

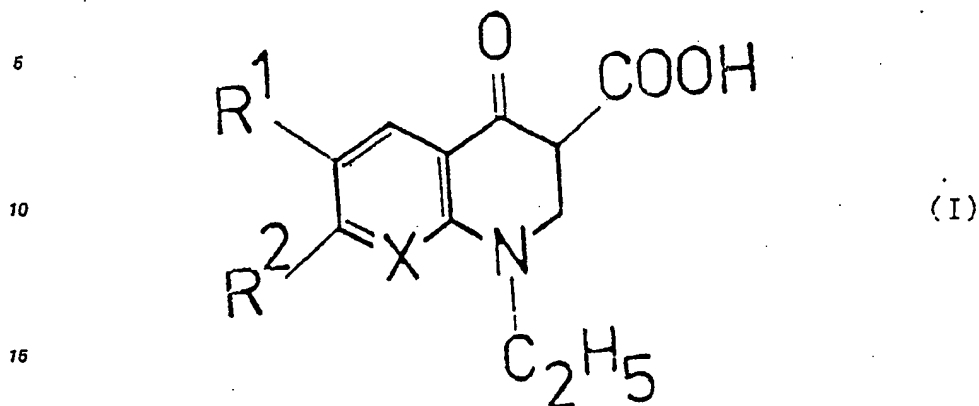
R³ and R⁴ are hydrogen; or

R³ and R⁴ together represent an additional chemical bond,

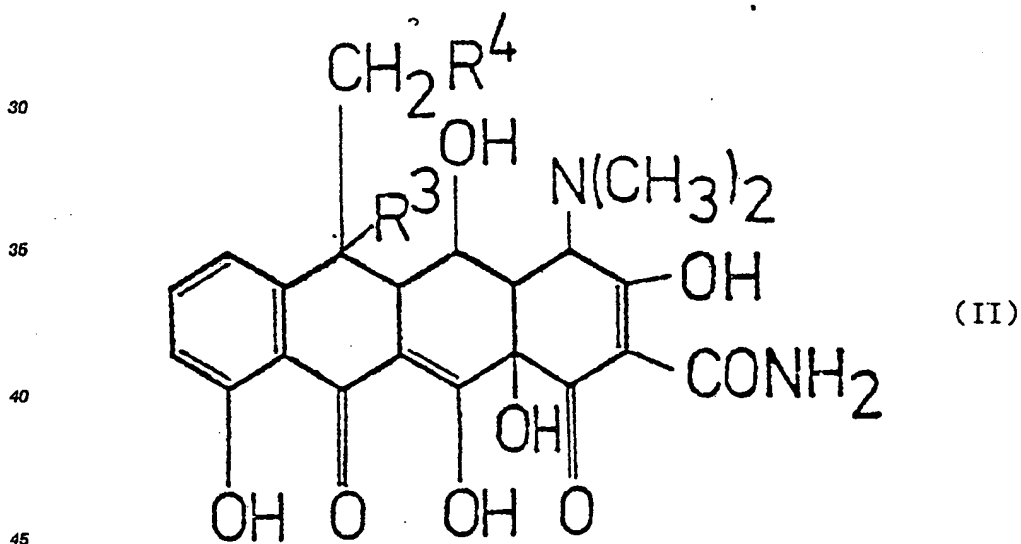
in an 1:1 to 1:20 ratio of the compound of the general formula (I) to the compound of the general formula (II), optionally in an admixture with an amount required to 100 % by weight of an inert, solid or liquid carrier such as magnesium carbonate, magnesium stearate, starch, talc, cyclodextrine or water and other additives such as filling, disintegrating, sliding and emulsifying agents.

PHARMACEUTICAL COMPOSITION

This invention relates to synergistic, anti-microbial pharmaceutical compositions containing a quinolinecarboxylic acid derivative or a naphthyridine-carboxylic acid derivative of the general formula (I),



20 wherein
 X is carbon or nitrogen;
 R² is hydrogen or fluorine;
 R² is methyl, piperazino or methylpiperazino group; or
 R¹ and R² together are a methylenedioxy group;
 25 and a tetracycline derivative of the general formula (II),



wherein
 R³ and R⁴ are hydrogen; or
 R³ and R⁴ together represent an additional chemical bond,
 50 as active ingredients.

In an other aspect of the invention, there is provided a process for the preparation of these compositions.

In the antimicrobial therapy, a continuous combat exists between the adaptation capability of microorganisms (development of resistance) and the preparation of novel drugs.

In the case of novel drugs, the adaptation capability, i.e. the resistance usually develops within a shorter or longer period. It can be expected that the development of the resistance becomes particularly rapid when the new substance is a derivative of a drug previously used for a long time since in this case, the resistance developed to the starting compound will of course more rapidly be modified for the derivatives.

5 The development of the resistance can be delayed by the simultaneous administration, i.e. combination of several active compounds whereby the metabolism of the microorganisms is attacked at several points at the same time. This results that the resistance of the microorganisms to the combination hardly or long afterwards develops thus, the desired "microbicidal" (killing) effect is strengthened.

In the antimicrobial therapy, nalidixic acid has been used for a long time as active ingredient. It was 10 published that from its derivatives, norfloxacin (Belgian patent specification, No. 863,429) and perfloxacin (Belgian patent specifications Nos. 870,576 and 870,917) show a highly favourable effect on gram-negative pathogens whereas their effect on gram-positive pathogens is more moderate.

Tetracycline is also a long-known antimicrobial substance. Out of its derivatives, doxycycline has a very favourable effect on gram-positive pathogens and a moderate effect on gram-negative ones.

15 The aim of the invention is to prepare broad-spectrum pharmaceutical compositions by combining these two types of active substances and thereby to inhibit the development of resistance.

When combining tetracycline derivatives with the quinoline-carboxylic acid derivatives or naphthyridine-carboxylic acid derivatives of the general formula (I), it was surprisingly observed that, in addition to the realization of the aim of the invention, a high-level synergistic action of these two types of active substances 20 occurred, whereby the effective doses could strongly be decreased with the important advantages of less side-effects and a cheaper therapy.

Thus, the present invention relates to the preparation of a synergistic, antimicrobial pharmaceutical composition containing a quinolinecarboxylic acid derivative or a naphthyridinecarboxylic acid derivative of the general formula (I), wherein

25 X is carbon or nitrogen;

R¹ is hydrogen or fluorine;

R² is methyl, piperazino or methylpiperazino group; or

R¹ and R² together are a methylenedioxy group; and a tetracycline derivative of the general formula (II), wherein

30 R³ and R⁴ are hydrogen; or

R³ and R⁴ together represent an additional chemical bond,

as active ingredients, which comprises mixing together 0.01 to 50 % by weight of a quinolinecarboxylic acid derivative or a naphthyridinecarboxylic acid derivative of the general formula (I), wherein X, R¹ and R² are the same as defined above and 0.01 to 95 % by weight of a tetracycline derivative of the general formula 35 (II), wherein R³ and R⁴ are the same as defined above while maintaining the ratio of the compound of the general formula (I) to the compound of the general formula (II) as 1:1 to 1:20, and optionally inert, solid or liquid carriers, preferably magnesium carbonate, magnesium stearate, starch, talc, cyclodextrin or water as well as binding, disintegrating, emulsifying, sliding agents and lubricants as additives and formulating them in a known way to a pharmaceutical composition suitable for therapeutical application.

40 In the process of the invention, preferably a compound of the general formula (I), wherein X, R¹ and R² are as defined above, suitably norfloxacin (1,4-dihydro-1-ethyl-6-fluoro-4-oxo-7-piperazinoquinoline-3-carboxylic acid) and doxycycline (4-dimethylamino-1,11-dioxo-6-methyl-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-2-naphthacenecarboxamid) may be used as active ingredients of the combination.

45 Similarly, oxolinic acid (1,4-dihydro-1-ethyl-6,7-methylenedioxy-4-oxoquinoline-3-carboxylic acid) and methacycline (4-dimethylamino-1,11-dioxo-6-methylene-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-2-naphthacenecarboxamide) may preferably be used in the combination according to the invention.

According to a preferred embodiment of the invention, the active ingredients are used in an 1:1 ration. If 50 desired, the compositions may contain also other active ingredients, (such as antibiotics, chemotherapeutics or the like).

The pharmaceutical compositions according to the invention may be formulated in solid forms, such as granulates, tablets, capsules, dragées and suppositories, semisolid forms, such as ointments and the like or liquid forms, such as injectable solutions, emulsions or suspensions. Preferably gels, ointments, dusting 55 powders for wounds, injectable solutions and suspensions as well as the combinations of powder and solvent ampoules are prepared.

Depending on the formulation, magnesium carbonate, magnesium stearate, starch, talc and water as commonly used carriers, cyclodextrin as a novel carrier as well as other additives such as vehicles,

disintegrating, sliding and emulsifying agents may be used.

The compositions according to the invention may be administered in oral, parenteral or rectal route or may be topically used.

The orally useful compositions are e.g. granulates, tablets, capsules or dragées. Parenterally useful compositions are e.g. the aqueous emulsions, suspensions or solutions. Ointments, aqueous or oily emulsions and suspensions as well as sprays may topically be applied.

The pharmaceutical compositions containing the synergistic active ingredient combination may be used in the veterinary medicine, too, e.g. in the form of a powder mixed to the fodder, or in the form of a solution added to the drinking fluid of the animals. For this purpose, compositions containing a combination of oxolinic acid and methacycline are preferably used.

The *in vitro* biological activity of the compositions according to the invention are shown in Tables I to V.

The international resistant and/or polyresistant human-pathogenic and/or veterinary-pathogenic microorganisms used in these investigations were as follows.

- 1.) *Vibrio parahaemolyticus* CCM.5938.
- 2.) *Pseudomonas fluorescens* CCM.2115.
- 3.) *Pseudomonas pictorum* CCM.284.
- 4.) *Pseudomonas acidovorans* CCM.283.
- 5.) *Proteus vulgaris* CCM.1799.
- 6.) *Proteus mirabilis* CCM.1944.
- 7.) *Shigella sonnei* CCM.1373.
- 8.) *Salmonella typhimurium* CCM.5445.
- 9.) *Salmonella cholerae suis* CCM.5438.
- 10.) *Escherichia coli* DSM.30038.
- 11.) *Escherichia coli* CCM.5863.
- 12.) *Escherichia coli* CCM.5172.
- 13.) *Klebsiella pneumoniae* CCM.1848.
- 14.) *Serratia marcescens* CCM.303.
- 15.) *Pasteurella multocida* CCM.5419.
- 16.) *Staphylococcus aureus* CCM.885.
- 17.) *Staphylococcus aureus* CCM.2317.
- 18.) *Staphylococcus aureus* CCM.2326.
- 19.) *Streptococcus agalactiae* CCM.5534.
- 20.) *Streptococcus disgalactiae* CCM.5548.
- 21.) *Bacillus subtilis* ATCC.6633.
- 22.) *Micrococcus flavus* ATCC.10240.
- 23.) *Bacillus licheniformis* CCM.2182.
- 24.) *Bacillus licheniformis* CCM.2205.
- 25.) *Pseudomonas putrefaciens* Sz-III-156.
- 26.) *Pseudomonas fluorescens putida* M-III-21.
- 27.) *Pseudomonas fluorescens putida* K-I-86.

Abbreviations used hereinabove and hereinafter are as follows:

ATCC = The American Type Culture Collection

CCM = Czechoslovak Collection of Microorganisms

DSM = Deutsche Sammlung für Mikroorganismen

μg/ml = microgram/millilitre

The investigations were carried out on a Difco Bouillon medium (in the case of bacteria) or on a modified Difco Bouillon medium (in the case of vibrios).

The inoculation was made with a germ number of 5×10^5 /ml. The incubation lasted 24 hours at 37 °C.

It is obvious from the data of the Tables that, due to the synergistic effect, from the combination a part and in some cases even a fraction of the amounts of the active ingredients, (as calculated for their individual activity), is sufficient to achieve an identical effect.

Table I

Combination of Nalidixic Acid with Doxycycline									
		MIC value µg/ml				Decrease of the MIC value by the combination %			Effect, %
		Nal	Dox	Combination		Nal	Dox	Nal + Dox	
				Nal	Dox				
<i>Vibrio p. haemolyticus</i>	CCM.5938.	5	0.5	0.5	0.25	10	50	30	40
<i>Pseudomonas pictorum</i>	CCM.284.	25	0.75	2.5	0.075	10	10	10	80
<i>Proteus vulgaris</i>	CCM.1799.	50	10	5	2.5	10	25	18.5	85
<i>Proteus mirabilis</i>	CCM.1944.	10	25	2.5	5	25	20	22.5	55
<i>Salmonella typhimurium</i>	CCM.5445.	50	10	5	5	10	50	30	40
<i>Salmonella cholerae suis</i>	CCM.5438.	25	0.5	2.5	0.05	10	10	10	80
<i>Escherichia coli</i>	DSM.30038.	50	5	5	0.5	10	10	10	80
<i>Escherichia coli</i>	CCM.5863.	25	5	2.5	0.5	10	10	10	80
<i>Escherichia coli</i>	CCM.5172.	50	2.5	5	0.5	10	20	15	70
<i>Pasteurella multocida</i>	CCM.5419.	50	0.25	5	0.025	10	10	10	80
<i>Staphylococcus aureus</i>	CCM.2317.	100	0.25	10	0.05	10	20	25	70
<i>Staphylococcus aureus</i>	CCM.2326.	100	0.25	10	0.075	10	30	20	60
<i>Streptococcus disgalactiae</i>	CCM.5548.	75	0.5	10	0.25	13.3	50	31.6	36.7
Abbreviations: Nal = nalidixic acid Dox = doxycycline									

Table II

Combination of Oxolinic acid with Doxycycline											
		MIC value µg/ml				Decrease of the MIC value by the combination %				Effect, %	
		Ox	Combination		Ox	Dox	Ox + Dox	Additive	Synerg.		
			Dox	Ox							
<i>Vibrio p-Haemolyticus</i>	CCM.5939.	1	0.5	0.1	0.25	10	50	30	60	40	
<i>Pseudomonas fluorescens</i>	CCM.2115.	10	0.5	0.25	0.25	2.5	50	26.3	52.5	47.5	
<i>Pseudomonas acidovorans</i>	CCM.283.	0.5	0.25	0.05	0.05	10	20	15	30	70	
<i>Pseudomonas pictorum</i>	CCM.284.	5	0.75	0.5	0.075	10	10	10	20	80	
<i>Shigella sonnei</i>	CCM.1373.	0.75	1	0.075	0.05	10	5	7.5	15	85	
<i>Escherichia coli</i>	CCM.5863.	5	5	0.75	0.75	15	15	15	30	70	
<i>Escherichia coli</i>	CCM.5172.	2.5	2.5	0.75	0.75	30	30	30	60	40	
<i>Staphylococcus aureus</i>	CCM.885.	25	1	2.5	0.1	10	10	10	20	80	
<i>Staphylococcus Aureus</i>	CCM.2317.	10	0.25	1	0.025	10	10	10	20	80	
<i>Bacillus subtilis</i>	ATCC.6633.	0.75	0.05	0.1	0.025	13.3	50	31.6	63.3	36.7	
<i>Bacillus cereus</i>	CCM.2010.	5	0.5	0.5	0.25	10	50	30	60	40	
Abbreviations: Ox = oxolinic acid											
Dox = doxycycline											

Table III

Combination of Norfloxacin with Doxycycline										
		MIC value $\mu\text{g/ml}$			Decrease of the MIC value by the combination %				Effect, %	
		Norf	Dox	Combination	Norf	Dox	Norf + Dox	Additive	Synerg.	
Vibrio p. haemolyticus	CCM.5938	0.5	0.5	0.25	0.1	50	20	35	70	30
Pseudomonas fluorescens	CCM.2115.	0.25	0.5	0.05	0.05	20	10	15	30	70
Pseudomonas pictor.	CCM.284.	0.75	0.75	0.1	0.25	13.3	33.3	23.3	46.6	53.4
Proteus vulg.	CCM.1799.	0.1	10	0.01	5	10	50	30	60	40
Shigella sonnei	CCM.1373.	0.1	1	0.01	0.5	10	50	30	60	40
Salmon. typhimur.	CCM.5445.	0.5	10	0.075	2.5	15	25	20	40	60
Salmon. Choleraesuis	CCM.5438.	0.5	0.5	0.05	0.05	10	10	10	20	80
Esch. Coll	DSM.30038.	0.1	5	0.025	0.75	25	15	20	40	60
Esch. coll	CCM.5863.	0.25	5	0.05	0.75	20	15	17.5	35	65
Past. multocida	CCM.5419.	0.5	0.25	0.05	0.025	10	10	10	20	80
Staph. aureus	CCM.885.	5	1	1	0.25	20	25	22.5	45	55
Strept. disgalact.	CCM.5548.	2.5	0.5	0.75	0.1	30	20	25	50	50
Abbreviations: Norf = Norfloxacin										
Dox = doxycycline										

Table IV

Combination of Pefloxacin with Doxycycline											
		MIC value µg/ml				Decrease of the MIC value by the combination %				Effect, %	
		Pefl	Dox	Combination		Pefl	Dox	Pefl + Dox	Additive	Synerg.	
				Pefl	Dox						
<i>Vibrio p. haemolyticus</i>	CCM.5938.	0.5	0.5	0.75	0.25	15	50	32.5	65	35	
<i>Proteus vulgaris</i>	CCM.1799.	0.5	10	0.1	0.05	20	0.5	10.2	20.5	79.5	
<i>Proteus mirabilis</i>	CCM.1944.	0.5	25	0.075	2.5	15	10	12.5	25	75	
<i>Shigella sonnei</i>	CCM.1373.	0.25	1	0.05	0.1	20	10	15	30	70	
<i>Salmon. typhimur</i>	CCM.5445.	2.5	10	0.5	2.5	20	25	22.5	45	55	
<i>Salmon. choleraesuis</i>	CCM.5438.	1	0.5	0.1	0.05	10	10	10	20	80	
<i>Esch. coli</i>	DSM.30038.	1	5	0.1	0.5	10	10	10	20	80	
<i>Esch. coli</i>	CCM.5863.	1	5	0.1	1	10	20	15	30	70	
<i>Klebs. pneumoniae</i>	CCM.1848.	0.75	2.5	0.075	1	10	40	25	50	50	
<i>Serratia marcesc.</i>	CCM.303.	0.5	25	0.1	10	20	40	30	60	40	
<i>Past. multocida</i>	CCM.5419.	0.25	0.25	0.05	0.05	20	20	20	40	60	
<i>Strept. disgalact.</i>	CCM.5548.	10	0.5	1	0.25	10	50	30	60	40	
<i>Pseud. putrefac.</i>	Sz-III-156.	0.25	0.5	0.05	0.075	20	15	17.5	35	65	
<i>Pseud. fluoresc. putida</i>	M-III-21.	2.5	2.5	0.5	0.75	20	30	25	50	50	
<i>Pseud. fluoresc. putida</i>	K-I-86.	2.5	0.25	0.05	0.05	2	20	11	22	78	
Abbreviations: Pefl = pefloxacin Dox = doxycycline											

Table V
Combination of Oxolinic acid with Methacycline

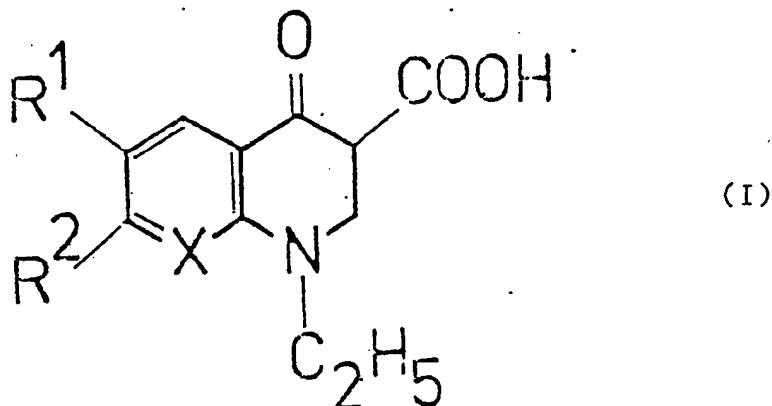
	MIC value /ug/ml		Decrease of the MIC value by the combination %				Effect, %		
	Combination		Ox	Methac	Ox	Methac	Ox + Methac	Additive	Synerg.
	Ox	Methac							
<i>Vibrio p. haemolyticus</i> CCM.5038.	1	1	0.25	0.1	25	10	17.5	35	65
<i>Pseud. fluoresc.</i> CCM.2115.	2.5	0.5	0.75	0.1	30	20	25	50	50
<i>Esch. coli</i> DSM.30038.	1	2.5	0.25	0.25	25	10	17.5	35	65
<i>Esch. coli</i> CCM.5863.	5	2.5	0.25	0.25	5	10	7.5	15	85
<i>Esch. coli</i> CCM.5172.	2.5	2.5	0.25	0.25	10	10	10	20	80

Abbreviations: Ox = oxolinic acid

Methac = methacycline

Claims

1. Synergistic, antimicrobial pharmaceutical composition which comprises 0.01 to 50 % by weight of a quinolinecarboxylic acid derivative or a naphthyridinecarboxylic acid derivative of the general formula (I),



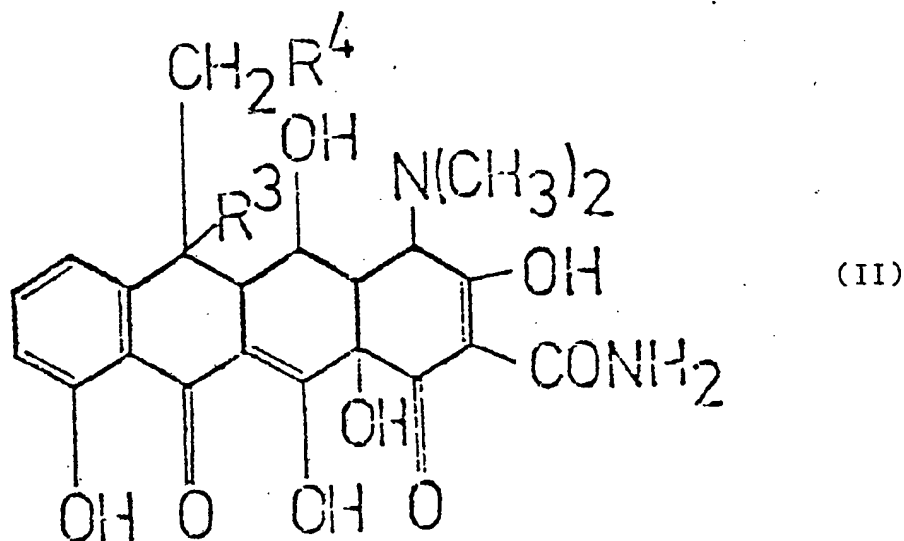
wherein

X is carbon or nitrogen:

R¹ is hydrogen or fluorine;

R² is methyl, piperazino or methylpiperazino group; or

R¹ and R² together are a methylenedioxy group; and 0.01 to 95 % by weight of a tetracycline derivative of the general formula (II),



wherein

R^3 and R^4 are hydrogen; or

R³ and R⁴ together represent an additional chemical bond,

In an 1:1 to 1:20 ratio of the compound of the general formula (I) to the compound of the general formula (II), optionally in an admixture with an amount required to 100 % by weight of an inert, solid or liquid carrier such as magnesium carbonate, magnesium stearate, starch, talc, cyclodextrine or water and other additives

such as filling, disintegrating, sliding and emulsifying agents.

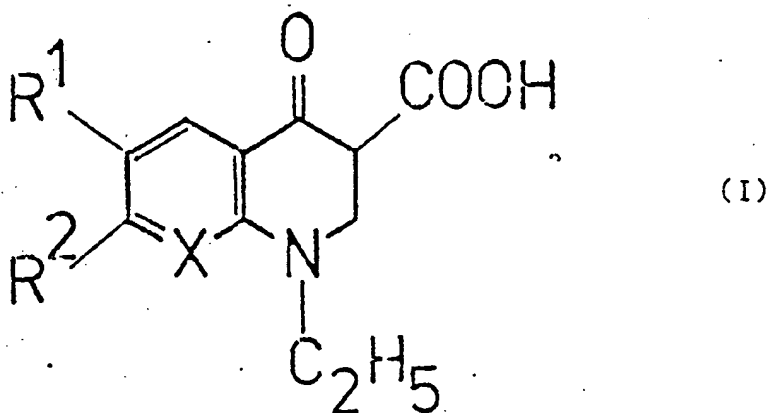
2. A composition as claimed in claim 1 which comprises a quinolinecarboxylic acid derivative or naphthyridinecarboxylic acid derivative of the general formula (I), wherein X, R¹ and R² are as defined in claim 1 and doxycycline (4-dimethylamino-1,11-dioxo-6-methyl-1,4,4a,5,5a,6,11,12,12a-octahydro-3,5,10,12,12a-pentahydroxy-2-naphthacenecarboxamide) as active ingredients.

3. A composition as claimed in claim 2, which comprises norfloxacin (1,4-dihydro-1-ethyl-6-fluoro-4-oxo-7-piperazinoquinoline-3-carboxylic acid) and doxycycline as active ingredients.

4. A composition as claimed in claim 1, which comprises oxolinic acid (1,4-dihydro-1-ethyl-6,7-methylene-dioxy-4-oxoquinoline-3-carboxylic acid) and methacycline (4-dimethylamino-1,11-dioxo-6-methylene-1,4,4a,5,5a,6,11,12,12a-octahydro-3,5,10,12,12a-pentahydroxy-2-naphthacenecarboxamide) as active ingredients.

5. A composition as claimed in claim 1, which comprises the active ingredients in an 1:1 ratio.

6. Process for the preparation of a synergistic, antimicrobial, pharmaceutical composition containing a quinolinecarboxylic acid derivative or a naphthyridine-carboxylic acid derivative of the general formula (I),



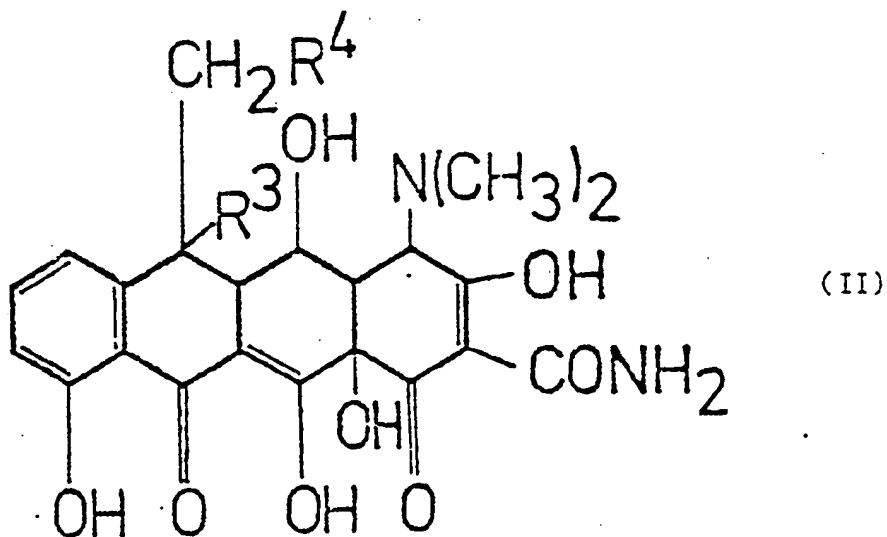
wherein

X is carbon or nitrogen;

R¹ is hydrogen or fluorine;

R² is methyl, piperazino or methylpiperazino group; or

R¹ and R² together are a methylenedioxy group; and a tetracycline derivative of the general formula (II),



wherein

R³ and R⁴ are hydrogen; or

R³ and R⁴ together represent an additional chemical bond,

as active ingredients, which comprises mixing together 0.01 to 50 % by weight of a quinolinecarboxylic acid
 5 derivative or a naphthyridinecarboxylic acid derivative of the general formula (I), wherein X, R¹ and R² are
 the same as defined above and 0.01 to 95 % by weight of a tetracycline derivative of the general formula
 (II), wherein R³ and R⁴ are the same as defined above while maintaining the ratio of the compound of the
 general formula (I) to the compound of the general formula (II) as 1:1 to 1:20, and optionally inert, solid or
 liquid carriers, preferably magnesium carbonate, magnesium stearate, starch, talc, cyclodextrin or water as
 10 well as binding, disintegrating, emulsifying, sliding agents and lubricants as additives and formulating them
 in a known way to a pharmaceutical composition suitable for therapeutical application.

15

20

25

30

35

40

45

50

55



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number

EP 88 30 6598

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
X	GB-A-2 035 800 (AUSONIA FARMACEUTICI SRL) * Page 1, line 5 - page 2, line 31 *	1-6	A 61 K 31/65 // (A 61 K 31/65 A 61 K 31:435) (A 61 K 31/65 A 61 K 31:495) (A 61 K 31/65 A 61 K 31:47)
X	FR-A-2 563 433 (ALLATORVOSTUDOMANYI EGYETEM) * Page 10, lines 1-29; claims 1-5 *	1-6	
			TECHNICAL FIELDS SEARCHED (Int. Cl.4)
			A 61 K
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 28-10-1988	Examiner BRINKMANN C.
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			